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**Cereal Grains with High Total Dietary Fiber and/or Resistant Starch  
Content and Their Preparation Thereof**

**FIELD OF THE INVENTION**

This invention relates to a process for preparing cereal grain having increased dietary fiber and/or resistant starch content and the process tolerant grain prepared thereby. Further, this invention provides high amylose grain with unusually high dietary fiber and resistant starch content. In particular, this invention involves the preparation of the improved grain by a combination of moisture and temperature conditions and further to use of the grain in the preparation of products containing starch.

**BACKGROUND OF THE INVENTION**

Cereal grains, including wheat, corn (or maize), rice, barley, rye, oats, and sorghum are basic food components of the human diet and contain important nutrients such as dietary fiber and starch.

The consumption of dietary fiber is particularly important to digestive health, and has been implicated as being useful for the prevention or treatment of certain diseases such as colon cancer. Generally, dietary fiber is defined to be the polysaccharides and remnants of plant materials that are resistant to hydrolysis (digestion) by human alimentary enzymes, including nonstarch polysaccharides, resistant starch, lignanin and minor components such as waxes, cutin and suberin. Because of the potential health benefits of foods rich in dietary fiber, many countries have recommended the increased consumption of such foods as a part of their dietary guidelines.

In addition to dietary fiber, grains also store energy in the form of starch. The amount of starch contained in cereal grain varies, but is generally

between 60 and 75% of the weight of the grain. Aside from its excellent nutritive value, starch is important because of its effect upon the physical properties of many of our foods, and is an important industrial commodity.

It is known that certain starch processing operations result in the transformation of starch into starch that is resistant to pancreatic amylase, known simply as resistant starch. Resistant starch resists digestion by pancreatic alpha-amylase and absorption in the small intestine, but passes into the large intestine where it is fermented by colonic microflora to short chain fatty acids and gases. Research literature indicates that this fermentation of resistant starch by colonic bacteria has numerous beneficial effects including improving colonic health and reducing the chance of developing diverticulosis and colon cancer. Further, as it is not utilized until it reaches the large intestine, where it is fermented to short chain fatty acids, resistant starch has a reduced caloric value and, in respect of these properties, has the benefits of dietary fiber.

Various methods have been reported for producing various types of resistant starch and focus on modifying starch that is already isolated from the cereal grain. These methods are discussed in U.S. Patent No. 5,593,503, the disclosure of which is incorporated herein by reference. In particular, U.S. Patent No. 5,593,503 discusses a process of treating a high amylose starch, isolated from the grain, having a moisture content of between 10 to 80% at temperatures of between 60° to 160° C thereby providing a granular starch with a total dietary fiber content of at least 12%.

The effect of heat treatment on the resistant starch content of cereal grain has been investigated. For example, Czarnecki et al. reported that the resistant starch content of barley, wheat, maize and triticale grain, as measured by digestibility of the component starch (sensitivity to amylolysis),

decreased over grain moisture contents of from 13 to 30% and heat treatment of between 100 to 130° C. Czarnecki et al., Effect of Heat Treatment on Carbohydrates and Protein in Cereal Grain. Biuletyn Informacyjny Przemysłu Paszowego 23(2): 1-16, 1984.

- 5            Generally, the heat/moisture treatment of grains has been reported to increase starch digestibility thereby indicating in decrease in resistant starch content. Alonso et al., Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans, Food Chemistry 68(2):159-165, 2000; Sagum, R. Arcot J.
- 10    Effect of domestic processing methods on the starch, non-starch polysaccharide and in vitro starch and protein digestibility of three varieties of rice with varying levels of amylose, Food Chemistry, 70 107-111, 2000; Yakovenko, V.A. Effect of hydrothermal processing of grain on physicochemical properties of starch in maize grits, Izvestiya Vysshikh Uchebnykh Zavedenii, Pishchevaya Tekhnologiya, 1971(6), 66-68; and
- 15    Yalanov. P.A. et al. Changes in the responsiveness to digestion of proteins and starch contained in the grain and grits of legumes during heat treatment, Vop. Pitan, 1973, (6), 75-9.

- In contrast, Barierguillot et al. reported that heat treatment of corn
- 20    kernels over a range of moisture contents had no effect on the digestibility of the component starch. The heat treatment included the use of a 2-step drying process affording a final grain moisture of 15% and heat treatment at temperatures varying from 80° to 160° C. Barierguillot et al, Effect of Heat Drying Temperature on the Nutritive Value of Corn in Chickens and Pigs,
- 25    Animal Feed Science and Technology 41(2): 149-159, 1993.

Unsuccessful attempts to increase the dietary fiber content of grain have been reported. For example, Fasina et al, subjected whole hulless and pearled barley to infrared heating at moisture contents of between 12.2% and 26.5% and surface temperatures of between 105 °C to 150 °C. No increase  
5 in the total dietary fiber content of the grain was found. Fasina, O.O., Tyler, R.T., Pickard, M.D., Zheng, G.H. Infrared heating of hulless and pearled barley, *Journal of Food Processing and Preservation*, 23(2), pg 135-151 (July 1999).

Surprisingly, it has now been discovered that the total dietary fiber  
10 content of cereal grains, particularly high amylose grains, can be significantly increased by heat treatment over a certain moisture range. Further, upon heat treatment, high amylose grains undergo an increase in resistant starch content as well as total dietary fiber content. Moreover, the cereal grains of this invention are uniquely process tolerant and may be processed directly  
15 into food products with little or no loss of dietary fiber and resistant starch content.

#### SUMMARY OF THE INVENTION

This invention relates to a process for preparing cereal grain having increased dietary fiber and/or resistant starch content and the process  
20 tolerant grain prepared thereby. Further, this invention provides high amylose grain with unusually high dietary fiber and resistant starch content. In particular, this invention involves the preparation of the improved grain by selected heat-moisture treatment and further to use of the grain in the preparation of products containing starch.

25 The method for preparing a grain with increased total dietary fiber content comprising heating a base grain having a total moisture content of from about 8% to about 85% by weight based on the dry weight of the grain, at a

temperature of from about 65 C to about 150 °C, under a combination of moisture and temperature conditions to provide a heat-treated grain having an increase in total dietary fiber content ("TDF") of at least 10%.

The improved grains of this invention are uniquely process tolerant  
5 and may be processed directly into food with little or no loss of dietary fiber and/or resistant starch content. This invention further includes the improved products which incorporate the heat-treated grain.

#### DETAILED DESCRIPTION OF THE INVENTION

This invention relates to a process for preparing cereal grain having  
10 increased dietary fiber and/or resistant starch content and the process tolerant grain prepared thereby. Further, this invention provides high amylose grain with unusually high dietary fiber and resistant starch content. In particular, this invention involves the preparation of the improved grain by a combination of moisture and temperature conditions and further to use of the  
15 grain in the preparation of products containing starch.

The term "total dietary fiber content" ("TDF") may include the polysaccharides and remnants of plant materials that are resistant to hydrolysis (digestion) by human alimentary enzymes, including nonstarch polysaccharides, resistant starch, lignanin and minor components such as  
20 waxes, cutin and suberin. As used herein, TDF is defined as measured by the weight of undigested material separated by filtration as described by the test described, *infra*.

The term "resistant starch (RS) " is defined as the sum of starch and starch degradation products that are not absorbed in the small intestine of  
25 healthy individuals and may be measured by a variety of tests known in the art. Resistant starch is defined herein as measured by treatment with pancreatic alpha amylase in the test described, *infra*.

The base grains used in preparing grains with unusually high TDF as well as containing alpha-amylase resistant starch may be any native grain derived from any native source. A native grain as used herein, is one as it is found in nature. Also suitable are grains derived from a plant obtained by standard breeding techniques including crossbreeding, translocation, inversion, transformation or any other method of gene or chromosome engineering to include variations thereof. In addition, grain derived from a plant grown from induced mutations and variations of the above generic composition which may be produced by known standard methods of mutation breeding are also suitable herein.

Typical sources for the base grains are cereals including wheat, corn or maize, rice, barley, rye, sorghum and particularly high amylose-containing varieties thereof, preferably corn. As used herein, the term "high amylose" is intended to include a grain containing starch composed of at least about 40% amylose by weight. Grains having a component starch with a high amylose content have been found to be most suitable for use in this invention, particularly grains of greater than 70% amylose content and most particularly greater than 90% amylose content.

It is well known that starch is composed of two fractions, the molecular arrangement of one being essentially linear and the other being highly branched. The linear fraction of starch is known as amylose and the branched fraction amylopectin. Starches from different grains are characterized by different relative proportions of the amylose and amylopectin components. Some plant species have been genetically developed which produce grain having starches which are characterized by a large preponderance of one fraction over the other. For instance, certain varieties of corn which normally contain about 22 to 28% amylose have been

developed which yield grain containing starch composed of over 40% amylose. These hybrid varieties have been referred to as high amylose. High amylose corn hybrids were developed in order to naturally provide grain containing starch of high amylose content and have been available  
5 commercially since about 1963.

Another useful base grain containing high amylose starch is extracted from a plant source having an amylose extender genotype, the component starch comprising less than 10% by weight amylopectin. This grain is derived from a plant breeding population, particularly corn, which is a genetic  
10 composite of germplasm selections and its starch comprises at least 75% by weight amylose, optionally at least 85% amylose (i.e., normal amylose) as measured by butanol fractionation/exclusion chromatography techniques. The starch further comprises less than 10%, by weight, optionally less than 5%, amylopectin and additionally from about 8 to 25% low molecular weight  
15 amylose. The grain is preferably derived from a plant having a recessive amylose extender genotype coupled with numerous amylose extender modifier genes. This grain and its method of preparation are described in U.S. Pat. No. 5,300,145, the specification of which is incorporated herein by reference.

20 The base grain of this invention also includes grains which have been partially processed by methods well known in the art including, for example, dry milled grains such as degerminated grits or kernels.

The improved grains of this invention are prepared hydrating a base grain to attain a moisture content of between about 8 to 85%, then heat  
25 treating said grain at temperatures of between 65° to 150° C, and drying the treated grain. The particular moisture content and heat treatment conditions which provide the improved grains ("heat-treated" grains) are dependent upon

the type and processing of the base grain used. In particular, partial processing of the base grain may reduce the necessary moisture content, temperature and time necessary to provide the improved grains of the present invention.

5           The total moisture or water content of the base grain to be heat-treated will typically be in a range of from about 8 to about 85% by weight, particularly about 15% to about 55%, more particularly from about 20% to about 45% by weight, and most particularly from about 20% to about 35%, based on the weight of the dry grain. The moisture uptake of the grain  
10       depends on the amount of water used during hydration, the temperature of the water, the degree of processing or milling of the grains and the botanical source. Hydration may be accomplished by a number of means known in the art, such as steeping in water. This relative level of moisture is maintained during a substantial portion of the heating step and is accomplished by  
15       methods known in art, for instance, by heating in a sealed container. After the heat treatment is complete, the grain may be dried further.

          The base grain with specified moisture content is typically heated at a temperature of from about 65 to 150°C, particularly from about 90 to 130°C, particularly between about 90 and about 125 °C and most particularly  
20       between about 90 and about 120 °C. The most desirable temperature may vary depending on the botanical source of the grain, degree of processing and the moisture content of the grain. Also, the time over which the grain is heated varies according to the base grain source, its degree of processing, moisture content, heating temperature as well as the level of total dietary fiber  
25       content desired. Typically, the heating time will be from about 0.5 to 24 hours and particularly from about 1 to 17 hours.



The most desired conditions for treating the grain to obtain a high level of total dietary fiber and/or resistant starch content are such that the granular structure of the starch is not completely destroyed. Under some conditions, such as at high moisture and high temperature, the component starch granule may be partially swollen but its crystallinity not completely destroyed. Accordingly, the term "granular starch" as used herein, means a starch which retains at least part of its granular structure thereby exhibiting some crystallinity, so that the granules are birefringent and the maltese cross is evident under polarized light according to the method described in US 5,849,090.

After heat treatment, the grain may be allowed to dry, for instance by air or belt drying, to reach an equilibrium moisture of between about 10 to about 15% by weight moisture. Other drying means may be used, including the use of fluid bed drying conditions.

The level of increase in dietary fiber content of the heat-treated (and dried) grain will vary depending on the processing conditions used as well as the particular base grain used. In particular, the grains will have at least a 10% increase, more particularly at least 30%, even more particularly at least 40%, and most particularly at least 50%.

Under certain conditions, the heat-treated grains of the present invention having an amylose content of greater than 40% may exhibit a desirable increase in resistant starch content as well as an increase in total dietary fiber content. In order to achieve an increase in both resistant starch and fiber content, the base high amylose grain must be hydrated to a moisture content of between about 20 to about 35% moisture followed by heat treatment at temperatures between about 90 °C to about 120 °C.

The treated grains of this invention may be used in foods directly or ground into flours and used in food to make products with high total dietary fiber content. In addition, the starch contained within the improved grains may be isolated from the improved grains by methods known to those in the art, including for example, by wet-milling the grains and extraction. These grains exhibit a remarkably high process tolerance characterized by the remarkably high retention of dietary fiber and resistant starch content of food into which the grain is processed into.

The heat-treated process tolerant grains of this invention can be identified by their component starches which exhibit a higher gelatinization onset temperature than corresponding, non-heat-treated grains. Further, the component starch of the heat-treated high-amylose grains of the present invention also show an increase in delta H as well as increases in the gelatinization onset temperature as compared to starch of the corresponding non-treated high-amylose grains.

The improved grains of this invention may be used in any food or beverage product (hereinafter collectively referred to as foods) to contribute to the total dietary fiber and resistant starch present as well as to reduce the caloric content. Typical food products include, but are not limited to, cereals such as ready-to-eat, puffed or expanded cereals and cereals which are cooked before eating; baked goods such as breads, crackers, cookies, cakes, muffins, rolls, pastries and other grain-based ingredients; pasta; beverages; fried and coated foods; snacks; and cultured dairy products such as yogurts, cheeses, and sour creams.

The amount of granular resistant starch and dietary fiber derived from the improved grain of this invention which can be added and used in any given food will be determined to a great extent by the amount that can be

tolerated from a functional standpoint. In other words, the amount of granular resistant starch and fiber used generally will be as high as will be acceptable in organoleptic evaluation of the food.

5 The improved grain of this invention may also be used to prepare a pharmaceutical or nutritional product, including but not limited to, prebiotic and synbiotic compositions, diabetic foods and supplements, dietetic foods, foods to control glycemic response, and tablets and other pharmaceutical dosage forms. A prebiotic composition is a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth, activity or  
10 both of one or a limited number of bacterial species already resident in the colon. A synbiotic composition may be a yogurt, capsule or other form of introduction into the host animal, including human beings, in which prebiotics are used in combination with a live microbial food supplement. The live microbial food supplement beneficially affects the host animal by improving its  
15 intestinal microbial balance.

Such live microbial food supplements may include, without limit, yeasts such as *Saccharomyces*, and bacteria such as the genera *Bifobacterium*, *Bacteriodes*, *Clostridium*, *Fusobacterium*, *Propionibacterium*, *Streptococcus*, *Enterococcus*, *Lactococcus*, *Staphylococcus*,  
20 *Peptostreptococcus* and *Lactobacillus*.

## EXAMPLES

The following examples are presented to further illustrate and explain the present invention and should not be taken as limiting in any regard. All  
25 parts and percentages are given by weight and all temperatures in degrees Celsius (°C ) unless otherwise noted.

The following ingredients were used throughout the examples.

Normal maize commercially available from National Starch and Chemical Company.

Long grain brown rice grain commercially available from Arrowhead Mills. (11%M).

5 Pearled barley grain commercially available from Arrowhead Mills (11.8%M).

High amylose barley, cultivar Glacier, available from Western Plant Breeders, Inc. (Boseman, Montana).

10 Whole wheat grain commercially available from Arrowhead Mills (11.9% M).

HYLON® V Grain commercially available from National Starch and Chemical Company.

HYLON® VII Grain commercially available from National Starch and Chemical Company.

15 Low amylopectin grain ("LAPS"), the grain and method of preparing such grain is disclosed in U.S. Patent No. 5,300,145.

Pancreatin, product #P-7545, commercially available from Sigma Chemical company.

The following test procedures were used throughout the examples.

20 A. Resistant Starch ("RS") Determination

A 0.1 M sodium phosphate buffer solution (pH = 6.9) was prepared from  $\text{Na}_2\text{HPO}_4$  and  $\text{NaH}_2\text{PO}_4$ . Porcine Pancreatin (1 gram, Sigma P7545) was added to the phosphate buffer solution (20 ml). After equilibrating for 20 minutes the suspension was centrifuged at 10,000 rpm for 5 minutes and the  
25 supernatant was kept on ice to afford the enzyme solution. Phosphate buffer was then added to anhydrous ground grain (5 grams, grain was ground to less than a 355 micron particle size) to a total weight of 42 grams and

equilibrated at 37° C for five minutes. The enzyme solution (8 grams) was then added to the mixture which was then incubated in a shaker bath at 37°C at 150 rpm for 6 hours. After digestion the mixture was then centrifuged at 10,000 rpm at 5° C for 10 minutes. The supernatant was discarded and the remaining solid air-dried overnight. The dried solid was weighed and its moisture content measured according to the procedure described herein, *infra*. The resistant starch content was calculated based on the remaining anhydrous grain content according to the following equation:  $RS\% = \text{remaining weight} \times (1 - \text{moisture}\%) / 5$ .

#### 10 B. Total Dietary Fiber Determination ("TDF")

The grain was ground to a particle size of less than 355 microns and added to a mixture of Termamyl® 120L solution (0.05 ml, commercially available from Novo-Nordisk)) and a 2-[N-morpholino]ethanesulfonic acid ("MES")/[hydroxymethyl]amino-methane ("TRIS") buffer solution (0.5 ml, commercially available from Aldrich), and deionized water added to bring the total sample weight to 50.00 grams. The sample was mixed on a vortex mixer for two minutes, then allowed to digest on a boiling water bath for two hours, mixing every 20 minutes to ensure a homogeneous solution. The solution was then placed on ice, filtered under vacuum and the resultant filter cake was washed with deionized water twice and air-dried overnight. After measuring the total weight and moisture content (procedure described herein, *infra*.) of the dried filter cake, the total dietary fiber content was calculated according to the following equation:  $TDF\% = \text{remaining weight} \times (1 - M\%) / 7.5$ .

#### 20 C. Moisture Content ("M%") Determination

25 The moisture content of the grains (ground to a particle size of less than 355 microns) were determined by the CENCO moisture balance

(balance set to 125 Watts on infrared, available from CSC Scientific Co., Inc.). To avoid charring of the samples, the temperature in the moisture balance was set to 70 °C. The readings obtained by this method are within 0.6% of absolute when checked against an oven moisture analysis method (AACC method 44-15A for corn grits).

#### D. Differential Scanning Calorimetry ("DSC") Measurements

Differential scanning calorimetry measurements were performed in a Perkin-Elmer DSC-7 (Norwalk, Ct, U.S.A.). The instrument was calibrated with indium. Samples (approximately 10 mg calculated dry weight of grain ground to a particle size of less than 355 microns) at a grain-to-water ratio of 1:3 were prepared and heated from 10°C to 160°C at a rate of 10°C/minute. The sample was quench-cooled to 10 °C and re-scanned from 10 °C to 160 °C at a rate of 10 °C/minute. An empty stainless-steel pan was used as a reference.

Delta H of the gelatinization of cereal grain is calculated by integration of the area under the endothermic peak in the first DSC curve obtained via the foregoing method.

#### E. Dry-milling or degermination procedure

Dry-milled or degerminated grains used in the following examples were dry-milled or degerminated according to the procedures described in Yuan, Jian and Flores, Roland, Laboratory Dry-Milling Performance of White Corn: Effect of Physical and Chemical Corn Characteristics, *Cereal Chem.* 7, pg 574, 3(5): 574- 578 (1996).

For example, cleaned dent corn was tempered to 24% moisture content by weight before degerminating. Tempered corn samples were then sent to a laboratory to be dehulled/degerminated. The samples were

then dried at 49°C to a 17% moisture content by weight over one hour. The dried grits and germs were then separated according to density by floating the grits and germs in a sodium nitrate solution having a specific density of 1.22 g/ml.

5    **Example 1 - Preparation of Grains with High TDF Content**

This example illustrates the procedure for preparing the treated grains of the present invention.

10        Kernels or grains of normal maize and high amylose-containing grain including Hylon® V, Hylon® VII and LAPS grains (50 grams each) were steeped in excess water for various periods of time to attain a moisture content of between 5 to about 55% by weight by the dry grain. The excess water was drained off, the kernels towel dried and their moisture content determined via the moisture balance test described above. Water uptake of the dry-milled corn kernels varied according to botanical source and degree  
15    of milling.

      The kernels were then heat-treated in an oven in a sealed jar for 0.5 to 24 hours, at temperatures of from about 65 to 150 °C. After heating, the kernels were air-dried overnight, then ground, then analyzed according to the above procedures for TDF and RS content. TDF and RS content varied  
20    according to the moisture content of the steeped grains and the temperature at which the heat treatment was conducted.

**Example 2 - Effect of Heat Treatment on the TDF Content of Grains from a Variety of Sources**

25        This example illustrates the desirable increase in TDF exhibited by grains obtained from a variety of sources upon heat treatment after hydration to attain a certain moisture.

Several whole grains (not degerminated) were steeped to between 24% to about 42% moisture content and heated between 80 °C to about 120 °C in order to evaluate the effect of the treatment upon the grain TDF and RS content. Grains tested included long grain rice, whole grain wheat, pearled  
 5 barley, high amylose barley, and Hylon® VII Grain which have amylose contents of 19%, 28%, 26%, 41%, and 73%, respectively by weight of the starch. The TDF values of the non-treated and heat-treated whole grains were measured as discussed in the procedure above and are reported in Table 1.

10

**TABLE 1**

Sample	TDF (%)	TDF (%) Treated	Conditions (%M, Temp. °C, duration)
Wheat	26.1	33.1	24.8%, 100°, 16 hrs
P. Barley	44.0	52.2	31.2%, 120°; 1.0 hr
Rice	28.1	38.8	25.6%, 110°, 16.0 hrs
High amylose barley	56.3	60.5	34.5%, 80°, 6 hrs
Hylon VII	63.4	81.1	41.4%, 110°, 17 hrs

The data reported in Table 1 demonstrates that upon moisture and heat-treatment according to the procedure of Example 1, the TDF content of  
 15 grains from a variety of sources may be desirably increased.

**Example 3 - Effect of Temperature and Moisture Content ("M%") on the Heat Treatment of Low Amylose Grains**

This example describes the temperature and moisture content  
 20 dependence of the TDF and RS content of heat-treated normal maize grains which had been dry-milled into grit and steeped to attain a moisture content of at least 13.1%.



TABLE 2

Properties of normal maize (grit) heat-treated at variable temperature and moisture content			
Time (hour)	M%	T <sub>oven</sub> (°C)	TDF (%)
0	13.1	--	12.1
16	13.1	110	11.9
16	17.5	100	13.9
16	35.9	80	17.7
16	30.0	100	23.3
16	35.9	110	12.4

As can be seen from the data reported in Table 2, in normal maize the TDF increases as moisture content increases over a temperature range of from greater than 75 °C to less than 110 °C and a moisture content of greater than 10 and less than 50%. Thus, the grains had to be hydrated before heat-treating in order to effect an increase in TDF content.

**Example 4 -- Effect of Temperature and Moisture Content ("M%") on the Heat Treatment of High Amylose Grains**

This example illustrates the effect of moisture and temperature on the desirable increase in TDF of heat-treated high amylose grains.

Samples of degerminated high amylose grains (Hylon® V, Hylon® VII, and LAPS Grains) were steeped to varying moisture contents and heat-treated at a variety of temperatures according to the method described in Example 1.

TABLE 3

Properties of Heat-treated High Amylose Grain				
Sample Grain	Time (hours)	M%	T <sub>oven</sub> (°C)	TDF (%)
<b>Hylon® V</b> sample 1	0	9.4	--	33.0
sample 2	16	9.4	80	39.3
sample 3	16	44.1	80	46.4
sample 4	16	43.6	110	54.2
<b>Hylon® VII</b> Sample 1	0	10.3	--	55.5
Sample 2	16	10.3	80	58.1
Sample 3	16	47.7	80	61.6
Sample 4	16	10.3	110	53.0
Sample 5	16.0	48.2	110	64.0
<b>LAPS</b> Sample 1	0	11.2	--	64.2
Sample 2	16.0	11.2	80	57.5
Sample 3	16.0	42.4	100	75.3
Sample 4	16.0	11.2	110	68.8
Sample 5	16.0	48.9	110	78.7

The results reported in Table 3 indicate that, under selected

5 conditions, TDF content of a grain having between 50 to 60% amylose may be increased to over 45%, a grain having between about 70-90% amylose may be increased to over 60% and a grain having over 90% amylose may be increased to over 75%.

**Example 5 – Effect of Treatment Time on the TDF of High Amylose Grain**

5

Under certain temperature and moisture content conditions, the increase in TDF values of a high amylose grain (degerminated grits, Hylon® V Grain) was directly related to the duration of the heat treatment.

**TABLE 4**

Properties of Heat-treated Grain Containing Hylon V® at constant Temperature over Time			
Time (hour	M%	T <sub>oven</sub> (°C)	TDF (%)
0	9.4	--	33.0
4.0	27.6	90	39.5
7.5	27.6	90	36.6
16.0	26.3	90	45.4

10

As shown in the Table 4, upon heat treatment over a period of 16 hours at 90 °C and a virtually constant moisture content, the TDF values for Hylon® V Grain increase from 33.0 to 45.5.

**Example 6 – Simultaneous increase in both TDF and RS of High Amylose Grains**

15

Under certain conditions the heat-treated high amylose grains of the present invention show a desirable increase in RS as well as TDF.

**TABLE 5**

Simultaneous Increase in TDF and RS of Heat-Treated High Amylose Grains (degerminated grits)					
Sample	TDF (%)	TDF (%) Treated	RS(%)	RS(%) Treated	Conditions (%M, Temp., duration)
Hylon®V Grain	33.0	40.2	71.1	75.3	31.9%, 100 °C, 16 hrs
Hylon®VII Grain	55.5	72.3	70.0	72.8	27.5%, 100 °C, 16 hrs
LAPS	64.2	77.4	65.2	71.5	24.2%, 100 °C, 16 hrs

The data in Table 5 show that a grain with an amylose content of greater than 40% (Hylon® V Grain) provided a TDF and RS increase of 7.2% and 4.2%, respectively; a grain with an amylose content of greater than 70% had a TDF and RS increase of 16.8% and 2.8%, respectively; and a very high amylose grain (LAPS) with an amylose content of greater than 90% gave a TDF and RS increase of 12.2% and 6.3%, respectively.

#### **Example 7 – Process Tolerance of Heat-treated Grains**

10 Low amylose and high amylose grains were heat-treated at 100 °C at various moisture contents (“M%”) for 16 hours and dried according to Example 1 and their onset temperatures measured in order to evaluate their tolerance for processing into foods. Delta H and onset temperatures were measured by DSC as described in Procedure D.

**TABLE 6**

<b>Process Tolerance of Heat-Treated Grains</b>					
<b>Sample</b>	<b>Treatment conditions</b>	<b>Onset T (°C) of non-treated grain</b>	<b>Onset T (°C) of heat-treated grain</b>	<b>Delta H (J/g) of non-treated grain</b>	<b>Delta H (J/g) of heat-treated grain</b>
Normal maize	30.0%M, 100°C, 16hrs	64.6	80.5	14.54	8.36
Hylon® V Grain	27.6%M, 100°C, 16hrs	67.3	85.6	8.31	13.56
Hylon®VII Grain	27.5%M, 100°C, 16hrs	70.1	91.7	9.02	18.45
LAPS	28.4%M, 100°C, 16hrs	82.2	92.3	3.22	12.40
High amylose barley	26.5%M, 80°C, 6 hrs	59.8	69.2	5.20	5.91
Rice	25.6%M, 110°C, 16hrs	72.6	81.9	19.12	14.45
Wheat	24.8%M, 100°C, 16hrs	57.4	67.7	7.02	6.00

As reported in Table 6, all the heat-treated and dried grains of the present invention demonstrate an improved process tolerance as reflected by the delay in onset temperature as compared to the non-treated grains.

In addition, the heat-treated high amylose grains of the present invention (high amylose barley, HYLON® V & VII, and LAPS) demonstrate increases in delta H as well as in onset temperature. Such a combination of increased delta H and increased onset temperature indicates a highly process tolerant grain which may be processed into foods without substantially losing the resistant starch and fiber content as the grains are processed into useful products via methods common in industrial and food applications.